agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

* * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 16:33:54 ON 05 DEC 2001

=> file registry
COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION
FULL ESTIMATED COST 0.15 0.15

FILE 'REGISTRY' ENTERED AT 16:34:00 ON 05 DEC 2001
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
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STRUCTURE FILE UPDATES: 3 DEC 2001 HIGHEST RN 373353-24-3
DICTIONARY FILE UPDATES: 3 DEC 2001 HIGHEST RN 373353-24-3

TSCA INFORMATION NOW CURRENT THROUGH July 7, 2001

Please note that search-term pricing does apply when conducting SmartSELECT searches.

Crossover limits have been increased. See HELP CROSSOVER for details.

Calculated physical property data is now available. See HELP PROPERTIES for more information. See STNote 27, Searching Properties in the CAS Registry File, for complete details:
http://www.cas.org/ONLINE/STN/STNOTES/stnotes27.pdf

=> s aminolevulininc acid ester/cn
L1 0 AMINOLEVULININC ACID ESTER/CN

=> s aminolevulininc acid ester
0 AMINOLEVULININC
5197361 ACID
3410113 ESTER
L2 0 AMINOLEVULININC ACID ESTER
(AMINOLEVULININC(W)ACID(W)ESTER)

=> s aminolevulininc acid/cn
L3 0 AMINOLEVULININC ACID/CN

=> s aminolevulinic acid
58 AMINOLEVULINIC
5197361 ACID
L4 58 AMINOLEVULINIC ACID
(AMINOLEVULINIC(W)ACID)

=> s 5 aminolevulinic acid
7004017 5
58 AMINOLEVULINIC
5197361 ACID

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L5          21 5 AMINOLEVULINIC ACID
               (5(W)AMINOLEVULINIC(W)ACID)

=> d 1-21

L5   ANSWER 1 OF 21  REGISTRY  COPYRIGHT 2001 ACS
RN   353911-86-1  REGISTRY
CN   5-AMINOLEVULINIC ACID SYNTHASE(DELTA-AMINOLEVULINATE
     SYNTHASE)(DELTA-ALA SYNTHETASE) PROTEIN (Sinorhizobium meliloti strain
     1021 gene hemA OR SMc03104) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank AL591792-derived protein GI 15076014
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1967 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5   ANSWER 2 OF 21  REGISTRY  COPYRIGHT 2001 ACS
RN   332973-21-4  REGISTRY
CN   5-Aminolevulinic acid synthase (Caulobacter crescentus gene CC1355)
     (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank AE005811-derived protein GI 13422706
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1967 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5   ANSWER 3 OF 21  REGISTRY  COPYRIGHT 2001 ACS
RN   225790-14-7  REGISTRY
CN   Synthase, aminolevulinate (Fusarium venenotum clone pZL3-3 gene hemA)
     (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   2: PN: US6033892 SEQID: 2 claimed protein
CN   5-Aminolevulinic acid synthase (Fusarium venenatum strain ATCC 20334
     clone pZL3-3 gene hemA)
CN   Synthase, aminolevulinate (Fusarium venenotum strain ATCC-20334 clone
     pZL3-3 gene hemA)
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, TOXLIT, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               2 REFERENCES IN FILE CA (1967 TO DATE)
               2 REFERENCES IN FILE CAPLUS (1967 TO DATE)
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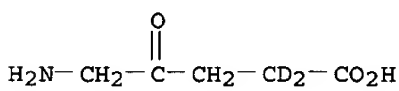
L5 ANSWER 4 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 216096-18-3 REGISTRY
CN Protein (Rickettsia prowazeki gene RP841) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **5-Aminolevulinic acid synthase hemA (Rickettsia prowazeki gene RP841)**
CN GenBank AJ235273-derived protein GI 3861366
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 5 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 207521-71-9 REGISTRY
CN Synthase, porphobilinogen (Pseudomonas aeruginosa clone pAYhemB gene hemB) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **5-aminolevulinic acid dehydratase (Pseudomonas aeruginosa clone pAYhemB gene hemB)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, TOXLIT

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

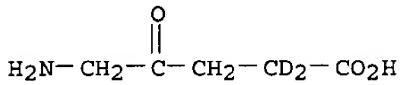
L5 ANSWER 6 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 187237-36-1 REGISTRY
CN Pentanoic-2,2-d2 acid, 5-amino-4-oxo- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **2,2-Dideutero-5-aminolevulinic acid**
MF C5 H7 D2 N O3
CI COM
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER, USPATFULL



2 REFERENCES IN FILE CA (1967 TO DATE)
2 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 7 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 187237-35-0 REGISTRY
CN Pentanoic-2,2-d2 acid, 5-amino-4-oxo-, hydrochloride (9CI) (CA INDEX

NAME)
OTHER NAMES:
CN **2,2-Dideutero-5-aminolevulinic acid hydrochloride**
MF C5 H7 D2 N O3 . Cl H
SR CA
LC STN Files: CA, CAPLUS, CASREACT, USPATFULL
CRN (187237-36-1)



2 REFERENCES IN FILE CA (1967 TO DATE)
2 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 8 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 168148-31-0 REGISTRY
CN Synthase, aminolevulinate (Rhodobacter sphaeroides strain H-5 gene hemA mutant H-5 reduced) (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Synthase, aminolevulinate (Rhodopseudomonas sphaeroides strain H-5 gene hemA mutant H-5 reduced)
OTHER NAMES:
CN **5-aminolevulinic acid synthase (Rhodobacter sphaeroides strain H-5 gene hemA mutant H-5 reduced)(E.C.2.3.1.37)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 9 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 168043-00-3 REGISTRY
CN Synthase, porphobilinogen (tomato precursor) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **5-Aminolevulinic acid dehydratase (tomato precursor)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS, TOXLIT

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 10 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 154248-52-9 REGISTRY

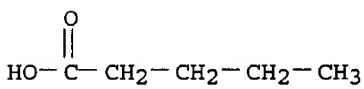
CN **DNA (tomato 5-aminolevulinic acid dehydratase cDNA plus flanks) (9CI)** (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN **Deoxyribonucleic acid (tomato 5-aminolevulinic acid dehydratase messenger RNA-complementary plus 5'- and 3'-flanking region fragment)**
OTHER NAMES:
CN GenBank L31367
FS NUCLEIC ACID SEQUENCE
MF Unspecified
CI MAN
SR GenBank
LC STN Files: AGRICOLA, BIOSIS, CA, CAPLUS, GENBANK, TOXLIT

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 11 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 145545-37-5 REGISTRY
CN Pentanoic acid, 5-amino-4-oxo-, pentanoate (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Pentanoic acid, compd. with 5-amino-4-oxopentanoic acid (1:1) (9CI)
OTHER NAMES:
CN **5-Aminolevulinic acid valerate**
MF C5 H10 O2 . C5 H9 N O3
SR CA
LC STN Files: CA, CAPLUS, TOXLIT, USPATFULL

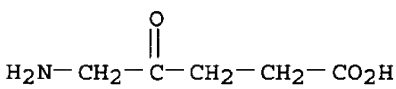
CM 1

CRN 109-52-4
CMF C5 H10 O2



CM 2

CRN 106-60-5
CMF C5 H9 N O3



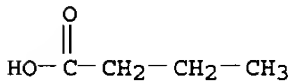
3 REFERENCES IN FILE CA (1967 TO DATE)
3 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 12 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 145545-36-4 REGISTRY
CN Pentanoic acid, 5-amino-4-oxo-, butanoate (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Butanoic acid, compd. with 5-amino-4-oxopentanoic acid (1:1) (9CI)

OTHER NAMES:
CN **5-Aminolevulinic acid butyrate**
MF C5 H9 N O3 . C4 H8 O2
SR CA
LC STN Files: CA, CAPLUS, TOXLIT, USPATFULL

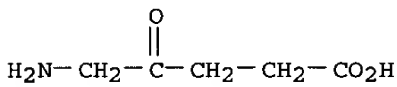
CM 1

CRN 107-92-6
CMF C4 H8 O2



CM 2

CRN 106-60-5
CMF C5 H9 N O3

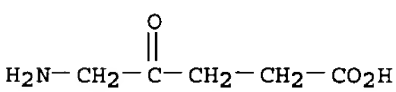


3 REFERENCES IN FILE CA (1967 TO DATE)
3 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 13 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 145545-35-3 REGISTRY
CN Pentanoic acid, 5-amino-4-oxo-, propanoate (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Propanoic acid, compd. with 5-amino-4-oxopentanoic acid (1:1) (9CI)
OTHER NAMES:
CN **5-Aminolevulinic acid propionate**
MF C5 H9 N O3 . C3 H6 O2
SR CA
LC STN Files: CA, CAPLUS, TOXLIT, USPATFULL

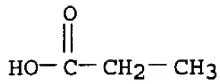
CM 1

CRN 106-60-5
CMF C5 H9 N O3



CM 2

CRN 79-09-4
CMF C3 H6 O2

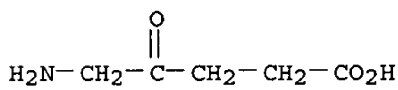


3 REFERENCES IN FILE CA (1967 TO DATE)
3 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 14 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 145545-34-2 REGISTRY
CN Pentanoic acid, 5-amino-4-oxo-, acetate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **5-Aminolevulinic acid acetate**
MF C5 H9 N O3 . C2 H4 O2
SR CA
LC STN Files: CA, CAPLUS, TOXLIT, USPATFULL

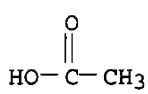
CM 1

CRN 106-60-5
CMF C5 H9 N O3



CM 2

CRN 64-19-7
CMF C2 H4 O2

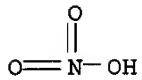


3 REFERENCES IN FILE CA (1967 TO DATE)
3 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 15 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 145545-32-0 REGISTRY
CN Pentanoic acid, 5-amino-4-oxo-, nitrate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **5-Aminolevulinic acid nitrate**
MF C5 H9 N O3 . H N O3
SR CA
LC STN Files: CA, CAPLUS, TOXLIT, USPATFULL

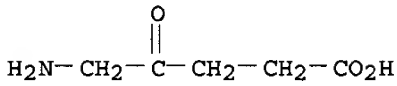
CM 1

CRN 7697-37-2
CMF H N O3



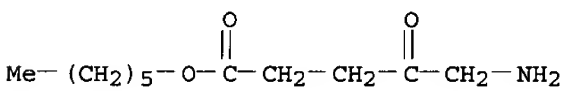
CM 2

CRN 106-60-5
CMF C5 H9 N O3



3 REFERENCES IN FILE CA (1967 TO DATE)
3 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 16 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 140898-97-1 REGISTRY
CN Pentanoic acid, 5-amino-4-oxo-, hexyl ester (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **5-Aminolevulinic acid hexyl ester**
CN Hexyl 5-aminolevulinate
FS 3D CONCORD
MF C11 H21 N O3
CI COM
SR CA
LC STN Files: BIOSIS, CA, CAPLUS, TOXCENTER, TOXLIT



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

17 REFERENCES IN FILE CA (1967 TO DATE)
18 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 17 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 9037-14-3 REGISTRY
CN Synthase, aminolevulinate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN .alpha.-Aminolevulinic acid synthase
CN .delta.-Aminolevulinate synthase
CN .delta.-Aminolevulinate synthetase
CN .delta.-Aminolevulinic acid synthase
CN .delta.-Aminolevulinic acid synthetase
CN .delta.-Aminolevulinic synthetase
CN 5-Aminolevulinate synthase
CN 5-Aminolevulinate synthetase
CN **5-Aminolevulinic acid synthase**
CN **5-Aminolevulinic acid synthetase**
CN ALA synthetase
CN Aminolevulinate synthase

CN Aminolevulinate synthetase
CN Aminolevulinic acid synthase
CN Aminolevulinic acid synthetase
CN Aminolevulinic synthetase
CN E.C. 2.3.1.37
CN Synthetase, aminolevulinate
DR 9037-20-1, 9039-12-7, 9047-02-3
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CAPLUS, EMBASE, TOXCENTER, TOXLIT, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
1400 REFERENCES IN FILE CA (1967 TO DATE)
1400 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 18 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 9036-37-7 REGISTRY
CN Synthase, porphobilinogen (9CI) (CA INDEX NAME)
OTHER NAMES:
CN .delta.-Aminolevulinate dehydrase
CN .delta.-Aminolevulinate dehydratase
CN .delta.-Aminolevulinic acid dehydrase
CN .delta.-Aminolevulinic acid dehydratase
CN .delta.-Aminolevulinic dehydratase
CN .gamma.-Aminolevulinic acid dehydratase
CN 5-Aminolevulinate dehydrase
CN 5-Aminolevulinate dehydratase
CN 5-Aminolevulinate hydrolyase
CN **5-Aminolevulinic acid dehydrase**
CN **5-Aminolevulinic acid dehydratase**
CN 5-Aminolevulinic dehydratase
CN 5-Levulinic acid dehydratase
CN Aminolevulinate dehydrase
CN Aminolevulinate dehydratase
CN Aminolevulinic acid dehydratase
CN Aminolevulinic acid dehydrogenase
CN Aminolevulinic dehydratase
CN E.C. 4.2.1.24
CN Porphobilinogen synthase
CN Porphobilinogen synthetase
DR 9023-42-1, 9037-15-4
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CAPLUS, CASREACT, CHEMCATS, CSCHEM, CSNB, EMBASE, IPA, NIOSHTIC, TOXCENTER, TOXLIT, USPATFULL

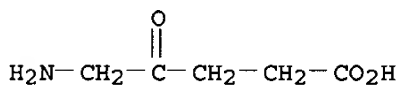
*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
1843 REFERENCES IN FILE CA (1967 TO DATE)
18 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
1845 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 19 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 9012-46-8 REGISTRY
CN Aminotransferase, aminolevulinate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN .gamma.,.delta.-Dioxovalerate aminotransferase
CN .gamma.,.delta.-Dioxovaleric acid transaminase
CN 4,5-Dioxovalerate aminotransferase

CN 4,5-Dioxovaleric acid transaminase
CN 4,5-Dioxovaleric transaminase
CN **5-Aminolevulinic acid transaminase**
CN Alanine-.gamma.,.delta.-dioxovalerate aminotransferase
CN Alanine-dioxovalerate aminotransferase
CN Alanine:4,5-dioxovalerate aminotransferase
CN Aminolevulinic acid transaminase
CN Dioxovalerate transaminase
CN E.C. 2.6.1.43
CN L-Alanine-4,5-dioxovalerate aminotransferase
CN L-Alanine:4,5-dioxovaleric acid transaminase
CN L-Alanine:dioxovalerate transaminase
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, EMBASE,
TOXCENTER, TOXLIT, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
53 REFERENCES IN FILE CA (1967 TO DATE)
53 REFERENCES IN FILE CAPLUS (1967 TO DATE)

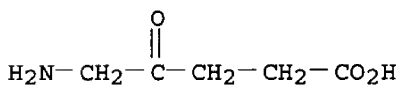
L5 ANSWER 20 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 5451-09-2 REGISTRY
CN Pentanoic acid, 5-amino-4-oxo-, hydrochloride (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Levulinic acid, 5-amino-, hydrochloride (8CI)
OTHER NAMES:
CN .delta.-Aminolevulinic acid hydrochloride
CN **5-Aminolevulinic acid hydrochloride**
CN Aminolevulinic acid hydrochloride
MF C5 H9 N O3 . Cl H
LC STN Files: BEILSTEIN*, BIOSIS, CA, CAPLUS, CASREACT, CHEMCATS, CHEMLIST,
CSCHEM, IPA, MRCK*, NIOSHTIC, RTECS*, TOXCENTER, TOXLIT, USPATFULL
(*File contains numerically searchable property data)
Other Sources: EINECS**
(**Enter CHEMLIST File for up-to-date regulatory information)
CRN (106-60-5)



81 REFERENCES IN FILE CA (1967 TO DATE)
81 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L5 ANSWER 21 OF 21 REGISTRY COPYRIGHT 2001 ACS
RN 106-60-5 REGISTRY
CN Pentanoic acid, 5-amino-4-oxo- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Levulinic acid, 5-amino- (8CI)
OTHER NAMES:
CN .delta.-Aminolevulinic acid

CN **5-Aminolevulinic acid**
CN Aminolevulinic acid
FS 3D CONCORD
MF C5 H9 N O3
CI COM
LC STN Files: ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, BEILSTEIN*, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CANCERLIT, CAOLD, CAPLUS, CASREACT, CBNB, CEN, CHEMCATS, CHEMINFORMRX, CHEMLIST, CIN, CSNB, DDFU, DIOGENES, DRUGU, EMBASE, IFICDB, IFIPAT, IFIUDB, IPA, MEDLINE, MRCK*, NAPRALERT, NIOSHTIC, PHAR, PIRA, PROMT, TOXCENTER, TOXLIT, USPATFULL
(*File contains numerically searchable property data)
Other Sources: EINECS**, NDSL**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

2894 REFERENCES IN FILE CA (1967 TO DATE)
34 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
2898 REFERENCES IN FILE CAPLUS (1967 TO DATE)
89 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

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=> s edta/cn
L6            1 EDTA/CN

=> s desferral/cn
L7            0 DESFERRAL/CN

=> s desferal
L8            2 DESFERAL

=> d l12
L12 NOT FOUND
The L-number entered has not been defined in this session, or it
has been deleted.  To see the L-numbers currently defined in this
session, enter DISPLAY HISTORY at an arrow prompt (=>).

=> d l8 1-2

L8   ANSWER 1 OF 2  REGISTRY  COPYRIGHT 2001 ACS
RN   14836-73-8  REGISTRY
CN   Iron, [N'-[5-[[4-[[5-[(acetyl-.kappa.O)(hydroxy-
     .kappa.O)amino]pentyl]amino]-1-(oxo-.kappa.O)-4-oxobutyl](hydroxy-
     .kappa.O)amino]pentyl]-N-(5-aminopentyl)-N-(hydroxy-
     .kappa.O)butanediamidato(3-)-.kappa.O1]- (9CI)  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   Butanediamide, N'-[5-[[4-[[5-(acetylhydroxyamino)pentyl]amino]-1,4-
     dioxobutyl]hydroxyamino]pentyl]-N-(5-aminopentyl)-N-hydroxy-, iron
complex
CN   Iron, [N'-[5-[[4-[[5-(acetylhydroxyamino)pentyl]amino]-1,4-
     dioxobutyl]hydroxyamino]pentyl]-N-(5-aminopentyl)-N-
     hydroxybutanediamidato(3-)]-
CN   Iron,
[N-[5-[3-[(5-aminopentyl)hydroxycarbamoyl]propionamido]pentyl]-3-[[5-
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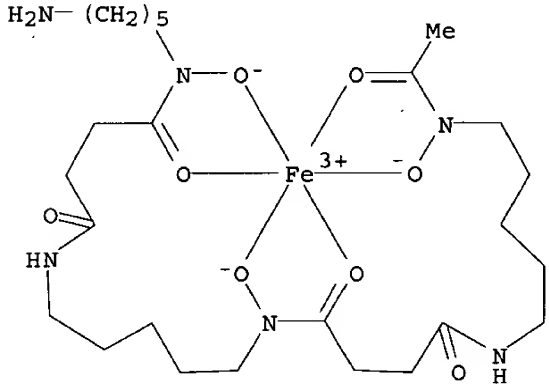
(N-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamato(3-)]- (7CI, 8CI)
OTHER NAMES:
CN **Desferal-iron**
CN Feroxamine
CN Ferric desferrioxamine
CN Ferrioxamine B
CN Ferrioxamine D
DR 12177-25-2, 15684-16-9
MF C25 H45 Fe N6 O8
CI CCS, COM
LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CANCERLIT, CAOLD, CAPLUS, CASREACT, DDFU, DRUGU, EMBASE, IPA, MEDLINE, TOXCENTER, TOXLIT, USPATFULL



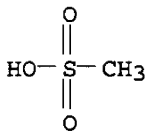
248 REFERENCES IN FILE CA (1967 TO DATE)
19 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
249 REFERENCES IN FILE CAPLUS (1967 TO DATE)
13 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

L8 ANSWER 2 OF 2 REGISTRY COPYRIGHT 2001 ACS
RN 138-14-7 REGISTRY
CN Butanediamide, N'-[5-[[4-[[5-(acetylhydroxyamino)pentyl]amino]-1,4-dioxobutyl]hydroxyamino]pentyl]-N-(5-aminopentyl)-N-hydroxy-, monomethanesulfonate (salt) (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Propionohydroxamic acid,
N-[5-[3-[(5-aminopentyl)hydroxycarbamoyl]propiona
mido]pentyl]-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]-, monomethanesulfonate (salt) (8CI)
CN Propionohydroxamic acid,
N-[5-[3-[(5-aminopentyl)hydroxycarbamoyl]propiona
mido]pentyl]-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]-, methanesulfonate (7CI)
OTHER NAMES:
CN Deferoxamine B mesylate
CN Deferoxamine mesylate
CN Deferrioxamine B methanesulfonate
CN Deferrioxamine methanesulfonate
CN **Desferal**
CN **Desferal mesylate**
CN Desferrioxamine B mesylate

CN Desferrioxamine B methanesulfonate
CN Desferrioxamine mesylate
CN Desferrioxamine methanesulfonate
AR 5115-09-3
DR 17688-38-9, 35908-62-4
MF C25 H48 N6 O8 . C H4 O3 S
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BEILSTEIN*, BIOBUSINESS, BIOSIS,
BIOTECHNO, CA, CAOLD, CAPLUS, CASREACT, CEN, CHEMCATS, CHEMLIST, CIN, CSCHEM, DIOGENES, EMBASE, GMELIN*, MRCK*, NIOSHTIC, PROMT, RTECS*, SYNTHLINE, TOXCENTER, TOXLIT, USAN, USPATFULL
(*File contains numerically searchable property data)
Other Sources: EINECS**
(**Enter CHEMLIST File for up-to-date regulatory information)

CM 1

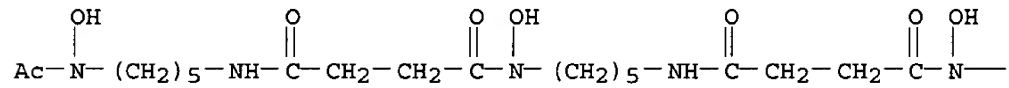
CRN 75-75-2
CMF C H4 O3 S



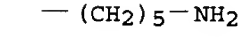
CM 2

CRN 70-51-9
CMF C25 H48 N6 O8

PAGE 1-A



PAGE 1-B

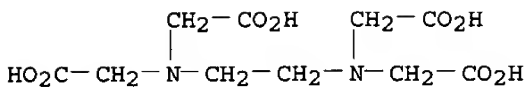


429 REFERENCES IN FILE CA (1967 TO DATE)
21 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
429 REFERENCES IN FILE CAPLUS (1967 TO DATE)
7 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

=> d l6

L6 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2001 ACS
RN 60-00-4 REGISTRY

CN Glycine, N,N'-1,2-ethanediylbis[N-(carboxymethyl)- (9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN Acetic acid, (ethylenedinitrilo)tetra- (8CI)
OTHER NAMES:
CN 3,6-Diazaoctanedioic acid, 3,6-bis(carboxymethyl)-
CN Acetic acid, 2,2',2'',2'''-(1,2-ethanediyldinitrilo)tetrakis-
CN Celon A
CN Celon ATH
CN Cheelox
CN Chelest 3A
CN Chemcolox 340
CN Clewat TAA
CN Complexon II
CN Dissolvine E
CN Edathamil
CN Edetic acid
CN **EDTA**
CN EDTA (chelating agent)
CN Endrate
CN Ethylenediamine-N,N,N',N'-tetraacetic acid
CN Ethylenediaminetetraacetic acid
CN Ethylenedinitrilotetraacetic acid
CN Gluma Cleanser
CN Havidote
CN ICRF 185
CN Metaquest A
CN N,N'-1,2-Ethanediyl-bis-N-(carboxymethyl)glycine
CN Nervanaid B acid
CN Nullapon B acid
CN Nullapon BF acid
CN Perma Kleer 50 acid
CN Quastal Special
CN Sequestrene AA
CN Sequestric acid
CN Sequestrol
CN Titriplex
CN Titriplex II
CN Trilon BS
CN Trilon BW
CN Versene
CN YD 30
FS 3D CONCORD
DR 13440-78-3, 20539-27-9, 94108-75-5, 26627-46-3, 30485-87-1, 30485-88-2, 30485-90-6, 32757-10-1, 161122-33-4
MF C10 H16 N2 O8
CI COM
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BEILSTEIN*, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CANCERLIT, CAOLD, CAPLUS, CASREACT, CBNB, CEN, CHEMCATS, CHEMINFORMRX, CHEMLIST, CIN, CSCHEM, CSNB, DDFU, DETHERM*, DIOGENES, DIPPR*, DRUGU, EMBASE, ENCOMPLIT, ENCOMPLIT2, ENCOMPPAT, ENCOMPPAT2, GMELIN*, HODOC*, HSDB*, IFICDB, IFIPAT, IFIUDB, IPA, MEDLINE, MRCK*, MSDS-OHS, NIOSHTIC, PDLCOM*, PIRA, PROMT, RTECS*, SPECINFO, TOXCENTER, TOXLIT, TULSA, ULIDAT, USAN, USPATFULL, VETU, VTB
(*File contains numerically searchable property data)
Other Sources: DSL**, EINECS**, TSCA**, WHO
(**Enter CHEMLIST File for up-to-date regulatory information)



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

19531 REFERENCES IN FILE CA (1967 TO DATE)
2720 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
19579 REFERENCES IN FILE CAPLUS (1967 TO DATE)
18 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

L12 ANSWER 1 OF 3 USPATFULL
AN 1999:48233 USPATFULL
TI Method for treating viral infections
IN Ben-Hur, Ehud, New York, NY, United States
Malik, Zvi, Emek Hefer, Israel
PA New York Blood Center, Inc., New York, NY, United States (U.S. corporation)
PI US 5895786 19990420
AI US 1996-646548 19960508 (8)
DT Utility
FS Granted
LN.CNT 445
INCL INCLM: 514/561.000
INCLS: 514/410.000; 514/185.000
NCL NCLM: 514/561.000
NCLS: 514/185.000; 514/410.000
IC [6]
ICM: A61K031-195
ICS: A61K031-40
EXF 514/410; 514/561; 514/185; 540/145; 562/567
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L12 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2001 ACS
AN 1999:690990 CAPLUS
DN 131:303402
TI Solution for diagnosing or treating tissue pathologies
IN Marti, Alexandre; Lange, Norbert; Zellweger, Matthieu; Wagnieres, George; Van Den Bergh, Hubert; Jichlinski, Patrice; Kucera, Pavel
PA Switz.
SO PCT Int. Appl., 18 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9953962	A1	19991028	WO 1999-CH163	19990422
W: CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
FR 2777782	A1	19991029	FR 1998-5425	19980422
FR 2777782	B1	20010518		
EP 1073472	A1	20010207	EP 1999-913060	19990422
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI FR 1998-5425	A	19980422		
WO 1999-CH163	W	19990422		

RE.CNT 2
RE
(1) Chang, S; Journal of Photochemistry and Photobiology B Biology 1997, V38(2-3), P114 CAPLUS
(2) Thomas, P; Phototherapie Dynamique Topique 1996, V15(5), P407

L12 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2001 ACS
AN 1997:745937 CAPLUS
DN 127:343405
TI Method for treating viral infections with 5-aminolevulinic acid and red light
IN Ben-Hur, Ehud; Malik, Zvi

PA New York Blood Center, Inc., USA; Bar-Ilan University
SO PCT Int. Appl., 27 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9741855	A1	19971113	WO 1997-US7811	19970507
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 5895786	A	19990420	US 1996-646548	19960508
	AU 9729365	A1	19971126	AU 1997-29365	19970507
	EP 904075	A1	19990331	EP 1997-923598	19970507
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2000510123	T2	20000808	JP 1997-540207	19970507
	US 6323012	B1	20011127	US 1999-234935	19990121
PRAI	US 1996-646548	A	19960508		
	WO 1997-US7811	W	19970507		

=>

12 ANSWER 1 OF 3 USPATFULL

DRWD . . . for 5 hours in growth medium with various mM concentrations of ALA alone (.smallcircle.) and in combination with 50 .mu.M **desferal** (.oval-solid.). The cells were then extracted with 1M HCL and protoporphyrin content was measured using spectrofluorimetery.

DRWD FIG. 2 represents HIV inactivation in U1 cells grown with 1 mM ALA and 50 .mu.M **desferal** for 5 hours followed by exposure to graded doses of red light at a fluence rate of 25 mW/cm.sup.2. HIV. . .

DETD . . . inhibits the conversion of protoporphyrin to haem by ferrochelatase in the virus-infected cells. Suitable iron chelating agents include EDTA and **desferal**. However, other iron chelating agents may be employed.

DETD . . . occurs when ALA was added at 1 mM to the growth medium. This accumulation is further enhanced approximately 2-fold when **desferal** was also added (FIG. 1). This is consistent with previous observations (Linuma, S., et al., Br. J. Cancer 70:21-28 (1994)) and is due to chelation of iron by **desferal**, which inhibits conversion of PP to haem by ferrochelatase. For subsequent experiments 1 mM ALA, 50 .mu.M **desferal** and 5 hour incubation was used to maximize the effect. FIG. 2 shows that under these conditions about 1.5 log.sub.10. . .

CLM What is claimed is:

. . . 3. The method of claim 1, wherein the iron chelating agent is selected from the group consisting of EDTA and **desferal**.

IT **60-00-4**, EDTA, biological studies **138-14-7**, Desferal
(aminolevulinic acid and red light and iron chelating agent for treatment of viral infections)

IT **106-60-5**, 5-Aminolevulinic acid
(aminolevulinic acid and red light for treatment of viral infections)

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                                                        ENTRY    SESSION
FULL ESTIMATED COST                                     86.95      87.10

FILE 'USPATFULL' ENTERED AT 16:37:07 ON 05 DEC 2001
CA INDEXING COPYRIGHT (C) 2001 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'CAPLUS' ENTERED AT 16:37:07 ON 05 DEC 2001
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2001 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 16:37:07 ON 05 DEC 2001
COPYRIGHT (C) 2001 BIOSIS(R)

=> d his

     (FILE 'HOME' ENTERED AT 16:33:54 ON 05 DEC 2001)

     FILE 'REGISTRY' ENTERED AT 16:34:00 ON 05 DEC 2001
L1             0 S AMINOLEVULININC ACID ESTER/CN
L2             0 S AMINOLEVULININC ACID ESTER
L3             0 S AMINOLEVULININC ACID/CN
L4            58 S AMINOLEVULINIC ACID
L5            21 S 5 AMINOLEVULINIC ACID
L6             1 S EDTA/CN
L7             0 S DESFERRAL/CN
L8             2 S DESFERAL

     FILE 'USPATFULL, CAPLUS, BIOSIS' ENTERED AT 16:37:07 ON 05 DEC 2001

=> s l5 or (aminolevulinic acid ester) or (amino levulinic acid ester)
L9       10423 L5 OR (AMINOLEVULINIC ACID ESTER) OR (AMINO LEVULINIC ACID
ESTER
               )

=> s l6 or etda
L10      31591 L6 OR ETDA

=> s l8 or desferal
L11       1742 L8 OR DESFERAL

=> s l9 and l10 and l11
L12          3 L9 AND L10 AND L11

=> d 1-3

L12  ANSWER 1 OF 3  USPATFULL
AN     1999:48233  USPATFULL
TI     Method for treating viral infections
IN     Ben-Hur, Ehud, New York, NY, United States
       Malik, Zvi, Emek Hefer, Israel
PA     New York Blood Center, Inc., New York, NY, United States (U.S.
       corporation)
PI     US 5895786              19990420
AI     US 1996-646548          19960508 (8)
DT     Utility
FS     Granted
LN.CNT 445
INCL   INCLM: 514/561.000
       INCLS: 514/410.000; 514/185.000
```

NCL NCLM: 514/561.000
NCLS: 514/185.000; 514/410.000
IC [6]
ICM: A61K031-195
ICS: A61K031-40
EXF 514/410; 514/561; 514/185; 540/145; 562/567
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L12 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2001 ACS
AN 1999:690990 CAPLUS
DN 131:303402
TI Solution for diagnosing or treating tissue pathologies
IN Marti, Alexandre; Lange, Norbert; Zellweger, Matthieu; Wagnieres, George; Van Den Bergh, Hubert; Jichlinski, Patrice; Kucera, Pavel
PA Switz.
SO PCT Int. Appl., 18 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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	FR 2777782	A1	19991029	FR 1998-5425	19980422
	FR 2777782	B1	20010518		
	EP 1073472	A1	20010207	EP 1999-913060	19990422
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PRAI	FR 1998-5425	A	19980422		
	WO 1999-CH163	W	19990422		

RE.CNT 2
RE
(1) Chang, S; Journal of Photochemistry and Photobiology B Biology 1997, V38(2-3), P114 CAPLUS
(2) Thomas, P; Phototherapie Dynamique Topique 1996, V15(5), P407

L12 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2001 ACS
AN 1997:745937 CAPLUS
DN 127:343405
TI Method for treating viral infections with 5-aminolevulinic acid and red light
IN Ben-Hur, Ehud; Malik, Zvi
PA New York Blood Center, Inc., USA; Bar-Ilan University
SO PCT Int. Appl., 27 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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	RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB,				

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            GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN,
            ML, MR, NE, SN, TD, TG
     US 5895786         A    19990420       US 1996-646548    19960508
     AU 9729365         A1   19971126       AU 1997-29365     19970507
     EP 904075          A1   19990331       EP 1997-923598    19970507
         R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
            IE, FI
     JP 2000510123      T2   20000808       JP 1997-540207    19970507
     US 6323012         B1   20011127       US 1999-234935    19990121
PRAI US 1996-646548     A    19960508
     WO 1997-US7811     W    19970507


=> d kwic 1

L12  ANSWER 1 OF 3  USPATFULL
DRWD   . . . for 5 hours in growth medium with various mM concentrations of
       ALA alone (.smallcircle.) and in combination with 50 .mu.M
       desferal (.oval-solid.). The cells were then extracted with 1M
       HCL and protoporphyrin content was measured using spectrofluorimetery.
DRWD   FIG. 2 represents HIV inactivation in U1 cells grown with 1 mM ALA and
       50 .mu.M desferal for 5 hours followed by exposure to graded
       doses of red light at a fluence rate of 25 mW/cm.sup.2. HIV.  . .
DETD   . . . inhibits the conversion of protoporphyrin to haem by
       ferrochelatase in the virus-infected cells. Suitable iron chelating
       agents include EDTA and desferal. However, other iron
       chelating agents may be employed.
DETD   . . . occurs when ALA was added at 1 mM to the growth medium. This
       accumulation is further enhanced approximately 2-fold when
       desferal was also added (FIG. 1). This is consistent with
       previous observations (Linuma, S., et al., Br. J. Cancer 70:21-28
       (1994)) and is due to chelation of iron by desferal, which
       inhibits conversion of PP to haem by ferrochelatase. For subsequent
       experiments 1 mM ALA, 50 .mu.M desferal and 5 hour incubation
       was used to maximize the effect. FIG. 2 shows that under these
       conditions about 1.5 log.sub.10.  . .
CLM    What is claimed is:
 . . . 3. The method of claim 1, wherein the iron chelating agent is
       selected from the group consisting of EDTA and desferal.

IT  60-00-4, EDTA, biological studies 138-14-7, Desferal
        (aminolevulinic acid and red light and iron chelating agent for
        treatment of viral infections)
IT  106-60-5, 5-Aminolevulinic acid
        (aminolevulinic acid and red light for treatment of viral infections)


=> d kwic 3

L12  ANSWER 3 OF 3  CAPLUS  COPYRIGHT 2001 ACS
IT   60-00-4, EDTA, biological studies 138-14-7,
     Desferal
     RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
        (aminolevulinic acid and red light and iron chelating agent for
        treatment of viral infections)
IT   106-60-5, 5-Aminolevulinic acid
     RL: BAC (Biological activity or effector, except adverse); THU
     (Therapeutic use); BIOL (Biological study); USES (Uses)
        (aminolevulinic acid and red light for treatment of viral infections)
```

```
=> d 3

L12  ANSWER 3 OF 3  CAPLUS  COPYRIGHT 2001 ACS
AN   1997:745937  CAPLUS
DN   127:343405
TI   Method for treating viral infections with 5-aminolevulinic acid and red
     light
IN   Ben-Hur, Ehud; Malik, Zvi
PA   New York Blood Center, Inc., USA; Bar-Ilan University
SO   PCT Int. Appl., 27 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
PI   WO 9741855         A1   19971113       WO 1997-US7811   19970507
         W:  AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
             DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC,
             LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,
             RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM,
             AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB,
             GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN,
             ML, MR, NE, SN, TD, TG
     US 5895786          A    19990420       US 1996-646548   19960508
     AU 9729365          A1   19971126       AU 1997-29365    19970507
     EP 904075           A1   19990331       EP 1997-923598   19970507
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, FI
     JP 2000510123       T2   20000808       JP 1997-540207   19970507
     US 6323012          B1   20011127       US 1999-234935   19990121
PRAI US 1996-646548      A    19960508
     WO 1997-US7811      W    19970507


=> d 2

L12  ANSWER 2 OF 3  CAPLUS  COPYRIGHT 2001 ACS
AN   1999:690990  CAPLUS
DN   131:303402
TI   Solution for diagnosing or treating tissue pathologies
IN   Marti, Alexandre; Lange, Norbert; Zellweger, Matthieu; Wagnieres, George;
     Van Den Bergh, Hubert; Jichlinski, Patrice; Kucera, Pavel
PA   Switz.
SO   PCT Int. Appl., 18 pp.
     CODEN: PIXXD2
DT   Patent
LA   French
FAN.CNT 1
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
PI   WO 9953962         A1   19991028       WO 1999-CH163    19990422
         W:  CA, JP, US
         RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
             PT, SE
     FR 2777782          A1   19991029       FR 1998-5425     19980422
     FR 2777782          B1   20010518
     EP 1073472          A1   20010207       EP 1999-913060   19990422
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        R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
            IE, FI
PRAI FR 1998-5425       A    19980422
     WO 1999-CH163      W    19990422
RE.CNT  2
RE
(1) Chang, S; Journal of Photochemistry and Photobiology B Biology 1997,
    V38(2-3), P114 CAPLUS
(2) Thomas, P; Phototherapie Dynamique Topique 1996, V15(5), P407


=> d jwuc 2

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or the STNGUIDE file for information on formats available in
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=> d kwic 2

L12  ANSWER 2 OF 3  CAPLUS  COPYRIGHT 2001 ACS
AB   The invention concerns a 5-aminolevulinic acid
     ester (I) soln. for producing a pharmaceutical prepn. useful for
     diagnosing and/or treating tissue and/or cell pathologies by local
     radiation exposure.  . .
IT   106-60-5D, 5-Aminolevulinic acid, esters 140898-97-1,
     Hexyl 5-Aminolevulinate
     RL: BAC (Biological activity or effector, except adverse); THU
     (Therapeutic use); BIOL (Biological study); USES (Uses)
        (soln. for diagnosing or treating tissue pathologies)
IT   60-00-4, Edta, biological studies   70-51-9, Deferoxamine
     138-14-7, Desferal   7647-14-5, Sodium chloride,
     biological studies
     RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
        (soln. for diagnosing or treating tissue pathologies)


=> d his

     (FILE 'HOME' ENTERED AT 16:33:54 ON 05 DEC 2001)

     FILE 'REGISTRY' ENTERED AT 16:34:00 ON 05 DEC 2001
L1             0 S AMINOLEVULININC ACID ESTER/CN
L2             0 S AMINOLEVULININC ACID ESTER
L3             0 S AMINOLEVULININC ACID/CN
L4            58 S AMINOLEVULINIC ACID
L5            21 S 5 AMINOLEVULINIC ACID
L6             1 S EDTA/CN
L7             0 S DESFERRAL/CN
L8             2 S DESFERAL

     FILE 'USPATFULL, CAPLUS, BIOSIS' ENTERED AT 16:37:07 ON 05 DEC 2001
L9         10423 S L5 OR (AMINOLEVULINIC ACID ESTER) OR (AMINO LEVULINIC ACID
ES
L10        31591 S L6 OR ETDA
L11         1742 S L8 OR DESFERAL
L12            3 S L9 AND L10 AND L11
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=> s l9 and l10
L13          75 L9 AND L10

=> s l9 and l11
L14          15 L9 AND L11

=> s l9 and solution
L15         179 L9 AND SOLUTION

=> s l15 and l10
L16           8 L15 AND L10

=> s l13 and solution
L17           8 L13 AND SOLUTION

=> s l17 not l12
L18           6 L17 NOT L12

=> d 1-18 ibib kwic

L18  ANSWER 1 OF 6  USPATFULL
ACCESSION NUMBER:        1998:124405  USPATFULL
TITLE:                   Method and compositions for enhancing aminolevulinic
                         acid dehydratase assay
INVENTOR(S):             Wong, Martin, Grayslake, IL, United States
                         Finley, David M., Spring Grove, IL, United States
PATENT ASSIGNEE(S):      Abbott Laboratories, Abbott Park, IL, United States
                         (U.S. corporation)

                              NUMBER        KIND    DATE
                         ---------------- ------ --------
PATENT INFORMATION:      US 5821074                19981013
APPLICATION INFO.:       US 1995-507168            19950726  (8)
RELATED APPLN. INFO.:    Continuation of Ser. No. US 1993-171121, filed on 21
                         Dec 1993, now abandoned
DOCUMENT TYPE:           Utility
FILE SEGMENT:            Granted
PRIMARY EXAMINER:        Schain, Howard E.
ASSISTANT EXAMINER:      Mohamed, Abdel A.
LEGAL REPRESENTATIVE:    Weinstein, David L.
NUMBER OF CLAIMS:        28
EXEMPLARY CLAIM:         1
LINE COUNT:              652
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     . . . enhances the recovery of lead during isolation of the lead
from
       interfering compounds by maintaining the lead in a sample
       solution and making the recovered lead available for detection
       by the assay. An enhancing reagent complexes with the lead isolated in
       the sample solution. The enhancer includes a chelator having a
       lead equilibrium binding constant in the range of about 4 log K to.  .
       .
SUMM   . . . demonstrating lead as a noncompetitive inhibitor of ALAD
       activity. The incubation mixtures contained DTT, ALAD and ALA in a
       buffer solution. The incubations were terminated by the
       addition of TCA containing HgCl.sub.2. The solution was
       centrifuged and the supernatant was added to modified Ehrlich's reagent
       in acetic acid and HClO.sub.4. The colored complex formed.  .  .
SUMM   . . . enhances the recovery of lead during isolation of the lead
from
```

interfering compounds by maintaining the lead in a sample **solution** and making the recovered lead available for detection by the assay.

SUMM The method includes isolating the lead in a sample **solution** from compounds which interfere with the lead assay. The recovery of the lead is enhanced in the sample **solution** and the lead is made available for assay. An ALAD enzyme is incubated in the sample **solution** in the presence of a substrate. The enzyme incubation step is stopped after a predetermined time interval. The extent of. . .

DETD . . . lead in the sample and then allows it to inhibit the ALAD enzyme activity. The term "neutralizing reagent" is the **solution** which brings the acidified supernatant sample to a neutral pH.

DETD . . . contemplates a method of enhancing the sensitivity and accuracy of a lead assay by isolating the lead in a sample **solution** from compounds which interfere with the lead assay. The pretreatment of the sample can be accomplished with conventional techniques such. . .

DETD . . . present in the sample during adjustment of the sample's pH to neutral. The enhancer recovers the lead in the sample **solution** and makes the lead available for continuing the assay.

DETD The assay continues by incubating an ALAD enzyme in the sample **solution** in the presence of a substrate such as ALA. The enzyme incubation step is stopped after a predetermined time interval.. . .

DETD . . . distilled water was adjusted to a pH 1.50 by adding an appropriate amount of concentrated HNO.sub.3. A 20 mM ZnCl.sub.2 **solution** was prepared by adding pH 1.50 distilled water to 0.0340 g. ZnCl.sub.2 for a final **solution** weight of 12.500 g. The **solution** was then thoroughly mixed.

DETD A **solution** of 200 ml 1.5 M BisTris was prepared by adding 62.70 g. of BisTris to HPLC grade distilled water to. . . to 7.30 with concentrated HNO.sub.3. The resulting volume was adjusted to the mark with distilled water. Similarly a 200 ml **solution** of 2.0 M BisTris was prepared by using 83.60 g. of BisTris. The pH was adjusted to pH 7.60 before. . .

DETD . . . adding 5 ml of ALAD containing 3.1 U/mg to 35 ml of 250 mM BisTris. The 250 mM BisTris diluent **solution** was prepared by adding 5.23 g. BisTris to 100 ml of HPLC grade distilled water and stirring. DTT was added. . . such glutathione, mercaptoethanol and cysteine can be used as a reducing agent instead of DTT. The pH of the diluent **solution** was adjusted to pH 7.0 by adding 50% NaOH. The diluted enzyme reagent was stored at 2.degree.-8.degree. C. under nitrogen. . .

DETD A 25 mM ALA and 10 uM ZnCl.sub.2 substrate **solution** was prepared by adding 0.210 g. ALA, 25 ul 20 mM ZnCl.sub.2 and 50 ml HPLC distilled water to a flask. After stirring, the substrate **solution** was stored at 2.8.degree. C. in the dark.

DETD . . . was prepared by adding 20.000 g of TCA, 0.1 M HgCl.sub.2 and HPLC grade distilled water to 200 ml. The **solution** was stirred and filtered at 0.80 um.

DETD For each enhancer, a neutralizing **solution** containing 0.5 M enhancer and 1.5 M BisTris was prepared by adding 7.5 ml of the 2 M Bis-Tris **solution** to following amounts of enhancers Na Citrate 1.470 gm; IDA 0.975 g; NTA 1.175 g; EGTA 1.900 g; Histidine 1.050. . . 1.840 g; EDTA 1.840 g; and PEN 0.745 g. After stirring, HPLC grade distilled water was added to each neutralizing **solution** to obtain a final volume of about 9.5 ml. Subsequently, the enhancer

solutions were vigorously stirred overnight. The neutralizing solutions.

. . were heated to about 70.degree. C. to complete dissolution and then cooled to room temperature. The pH of each neutralizing **solution** was then adjusted to pH 7.25 with either concentrated HNO.sub.3 or 50% NaOH.

DETD . . . and 40 ug/dl Pb.sup.+2 were dispensed in 24.5 ml amounts and were pretreated with 10.5 ml of the TCA pretreatment **solution**. Each sample was centrifuged for five minutes and the supernatants were saved. From each supernatant **solution** 180 ul was mixed by vortex with 180 ul of neutralizing buffer. From this neutralized supernatant **solution** 100 ul was added to 100 ul of the dilute enzyme reagent and mixed by vortex and incubated for 15. . .

DETD Subsequently 100 ul of the substrate **solution** was added, mixed by vortex and incubated for 30 min. in the water bath. The stop reagent was added in. . .

DETD . . . neutralizing reagent and the third is the enhancer. The enhancer may be kept separate or added to either the neutralizing **solution** or the acidified lead sample.

CLM What is claimed is:

1. A lead assay comprising the steps of: (a) providing an aqueous **solution** suspected of containing lead; (b) isolating said lead from said **solution** in such a manner that said lead remains in **solution**: (c) introducing to said **solution** of step (b) an enhancing reagent that combines with said lead and prevents said lead

from precipitating from said **solution**; followed by (d) introducing to said **solution** an enzyme the activity of which is inhibited in the presence of lead and a substrate for said enzyme: and. . .

5. A lead assay comprising the steps of: (a) providing an aqueous **solution** suspected of containing lead, said **solution** having been separated from compounds that are affected by the presence of lead, said **solution** further having been neutralized; (b) introducing into said **solution** a lead chelator having a lead equilibrium binding constant in the range of about 4 log K to about 13 log K; followed by (c) introducing into said **solution** (i) an enzyme the activity of which is inhibited by lead and (ii) a substrate which reacts with the enzyme; (d) incubating the **solution** of step (c): (e) stopping the incubation step after a predetermined interval; and (f) measuring the amount of lead as. . .

7. The lead assay of claim 5 wherein the method further includes neutralizing the sample **solution** before the enzyme incubating step.

12. The lead assay of claim 5 wherein the aqueous **solution** in step (b) is acidified and the lead chelator of step (c) is present in a neutralizing buffer such that performing step (c) results in bringing the acidified **solution** of step (b) to neutral pH.

15. The lead assay of claim 5 wherein the enzyme incubating step includes first incubating the sample **solution** in the presence of aminolevulinic acid dehydratase and subsequently incubating the sample **solution** in the presence of the substrate.

19. The lead assay of claim 5 wherein step (d) includes incubating the sample **solution** in the presence of a coloring reagent.

21. An aqueous lead assay reagent **solution** consisting essentially of neutralizing buffer and a lead chelator having a lead

binding constant in the range of about 4 log K to about 13 log K wherein
the concentration of the chelator in the **solution** is in the range of 0.5 mM to 500 mM.

22. The reagent **solution** of claim 21 wherein the chelator has a lead equilibrium binding constant in the range of about 6 log K. .

23. The reagent **solution** of claim 21 wherein the chelator is selected from the group consisting of N-benzyiminodiacetic acid, ethylenebis(oxyethylenenitrilo) tetraacetic acid, ethylenediaminetetraacetic acid,. . .

24. The reagent **solution** of claim 23 wherein the chelator is selected from the group consisting of 8-hydroxy-5-(2'-hydroxyphenylazo)quinoline, 8-hydroxy-5-(phenylazo)quinoline, N-(2-carboxyphenyl)iminodiacetic acid, N-(acetonyl) iminodiacetic acid,.

. .

25. The reagent **solution** of claim 21 wherein the chelator is selected from the group consisting of dihydroxyphenyl acetic acid, N-(2'-carboxyethyl) iminodiacetic acid, dihydroxybenzoic. . .

26. A lead assay reagent kit comprising: a container having a reagent **solution** consisting essentially of an aqueous neutralizing buffer and present therein a reagent which is capable of forming a compound or complex with lead such that adding the **solution** to an acidified aqueous sample containing lead will neutralize the aqueous sample while preventing precipitation of lead therefrom; a container.

.

IT 52-66-4, DL-Penicillamine **60-00-4**, EDTA, uses 67-42-5, EGTA 71-00-1, L-Histidine, uses 81-88-9 84-88-8, 8-Hydroxyquinoline-5-sulfonic acid 93-62-9, N-(2-Hydroxyethyl)-iminodiacetic acid **106-60-5**, Aminolevulinic acid 139-13-9, Nitrilotriacetic acid 142-73-4, Iminodiacetic acid 487-90-1, Porphobilinogen 994-36-5, Sodium citrate 3987-53-9, N-Benzyliminodiacetic acid 4408-64-4, Methyliminodiacetic acid 5961-85-3, Tris(2-carboxyethyl)phosphine **9036-37-7**
(automated lead assay in blood using disulfide enzyme)

L18 ANSWER 2 OF 6 USPATFULL

ACCESSION NUMBER:	97:42769 USPATFULL
TITLE:	Method and compositions for enhancing aminolevulinic acid dehydratase assay
INVENTOR(S):	Wong, Martin, Grayslake, IL, United States Finley, David M., Spring Grove, IL, United States
PATENT ASSIGNEE(S):	Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5631139		19970520
APPLICATION INFO.:	US 1995-507168		19950726 (8)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1993-171121, filed on 21 Dec 1993, now abandoned		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Schain, Howard E.		
ASSISTANT EXAMINER:	Mohamed, Abdel A.		
LEGAL REPRESENTATIVE:	Weinstein, David L.		
NUMBER OF CLAIMS:	28		

EXEMPLARY CLAIM: 1
LINE COUNT: 651
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB . . . enhances the recovery of lead during isolation of the lead from
interfering compounds by maintaining the lead in a sample **solution** and making the recovered lead available for detection by the assay. An enhancing reagent complexes with the lead isolated in the sample **solution**. The enhancer includes a chelator having a lead equilibrium binding constant in the range of about 4 log K to. . .
SUMM . . . demonstrating lead as a noncompetitive inhibitor of ALAD activity. The incubation mixtures contained DTT, ALAD and ALA in a buffer **solution**. The incubations were terminated by the addition of TCA containing HgCl.sub.2. The **solution** was centrifuged and the supernatant was added to modified Ehrlich's reagent in acetic acid and HClO.sub.4. The colored complex formed. . .
SUMM . . . enhances the recovery of lead during isolation of the lead from
interfering compounds by maintaining the lead in a sample **solution** and making the recovered lead available for detection by the assay.
SUMM The method includes isolating the lead in a sample **solution** from compounds which interfere with the lead assay. The recovery of the lead is enhanced in the sample **solution** and the lead is made available for assay. An ALAD enzyme is incubated in the sample **solution** in the presence of a substrate. The enzyme incubation step is stopped after a predetermined time interval. The extent of. . .
SUMM . . . lead in the sample and then allows it to inhibit the ALAD enzyme activity. The term "neutralizing reagent" is the **solution** which brings the acidified supernatant sample to a neutral pH.
SUMM . . . contemplates a method of enhancing the sensitivity and accuracy
of a lead assay by isolating the lead in a sample **solution** from compounds which interfere with the lead assay. The pretreatment of the sample can be accomplished with conventional techniques such. . .
SUMM . . . present in the sample during adjustment of the sample's pH to neutral. The enhancer recovers the lead in the sample **solution** and makes the lead available for continuing the assay.
SUMM The assay continues by incubating an ALAD enzyme in the sample **solution** in the presence of a substrate such as ALA. The enzyme incubation step is stopped after a predetermined time interval.. . .

DETD . . . distilled water was adjusted to a pH 1.50 by adding an appropriate amount of concentrated HNO.sub.3. A 20 mM ZnCl.sub.2 **solution** was prepared by adding pH 1.50 distilled water to 0.0340 g. ZnCl.sub.2 for a final **solution** weight of 12.500 g. The **solution** was then thoroughly mixed.
DETD A **solution** of 200 ml 1.5M BisTris was prepared by adding 62.70 g. of BisTris to HPLC grade distilled water to a. . . to 7.30 with concentrated HNO.sub.3. The resulting volume was adjusted to the mark with distilled water. Similarly a 200 ml **solution** of 2.0M BisTris was prepared by using 83.60 g. of BisTris. The pH was adjusted to pH 7.60 before adjusting. . .
DETD . . . adding 5 ml of ALAD containing 3.1 U/mg to 35 ml of 250 mM BisTris. The 250 mM BisTris diluent **solution** was prepared by adding 5.23 g. BisTris to 100 ml of HPLC grade distilled water and stirring. DTT was added. . . such glutathione, mercaptoethanol and cysteine can be used as a reducing agent instead of DTT. The pH of the

diluent **solution** was adjusted to pH 7.0 by adding 50% NaOH. The diluted enzyme reagent was stored at 2.degree.-8.degree. C. under nitrogen. . .

DETD A 25 mM ALA and 10 uM ZnCl.sub.2 substrate **solution** was prepared by adding 0.210 g. ALA, 25 ul 20 mM ZnCl.sub.2 and 50 ml HPLC distilled water to a flask. After stirring, the substrate **solution** was stored at 2.8.degree. C. in the dark.

DETD . . . TCA was prepared by adding 20.000 g of TCA, 0.1M HgCl.sub.2 and

HPLC grade distilled water to 200 ml. The **solution** was stirred and filtered at 0.80 um.

DETD For each enhancer, a neutralizing **solution** containing 0.5M enhancer and 1.5M BisTris was prepared by adding 7.5 ml of the 2M Bis-Tris **solution** to following amounts of enhancers Na Citrate 1.470 gm; IDA 0.975 g; NTA 1.175 g; EGTA 1.900 g; Histidine 1.050. .

.

1.840 g; EDTA 1.840 g; and PEN 0.745 g. After stirring, HPLC grade distilled water was added to each neutralizing **solution** to obtain a final volume of about 9.5 ml. Subsequently, the enhancer solutions were vigorously stirred overnight. The neutralizing solutions.

. . were heated to about 70.degree. C. to complete dissolution and then cooled to room temperature. The pH of each neutralizing **solution** was then adjusted to pH 7.25 with either concentrated HNO.sub.3 or 50% NaOH.

DETD . . . and 40 ug/dl Pb.sup.+2 were dispensed in 24.5 ml amounts and were pretreated with 10.5 ml of the TCA pretreatment **solution**. Each sample was centrifuged for five minutes and the supernatants were saved. From each supernatant **solution** 180 ul was mixed by vortex with 180 ul of neutralizing buffer. From this neutralized supernatant **solution** 100 ul was added to 100 ul of the dilute enzyme reagent and mixed by vortex and incubated for 15. . .

DETD Subsequently 100 ul of the substrate **solution** was added, mixed by vortex and incubated for 30 min. in the water bath. The stop reagent was added in. . .

DETD . . . neutralizing reagent and the third is the enhancer. The enhancer may be kept separate or added to either the neutralizing **solution** or the acidified lead sample.

CLM What is claimed is:

1. A lead assay comprising the steps of: (a) providing an aqueous **solution** suspected of containing lead; (b) isolating said lead from said **solution** in such a manner that said lead remains in **solution**; (c) introducing to said **solution** of step (b) an enhancing reagent that combines with said lead and prevents said lead

from precipitating from said **solution**; followed by (d) introducing to said **solution** an enzyme the activity of which is inhibited in the presence of lead and a substrate for said enzyme; and. . .

5. A lead assay comprising the steps of: (a) providing an aqueous **solution** suspected of containing lead, said **solution** having been separated from compounds that are affected by the presence of lead, said **solution** further having been neutralized; (b) introducing into said **solution** a lead chelator having a lead equilibrium binding constant in the range of about 4 log K to about 13 log K; followed by (c) introducing into said **solution** (i) an enzyme the activity of which is inhibited by lead and (ii) a substrate which reacts with the enzyme; (d) incubating the **solution** of step (c); (e) stopping the incubation step after a predetermined interval; and (f) measuring the amount of lead as. . .

7. The lead assay of claim 5 wherein the method further includes neutralizing the sample **solution** before the enzyme incubating step.

12. The lead assay of claim 5 wherein the aqueous **solution** in step (b) is acidified and the lead chelator of step (c) is present in a neutralizing buffer such that performing step (c) results in bringing the acidified **solution** of step (b) to neutral pH.

15. The lead assay of claim 5 wherein the enzyme incubating step includes first incubating the sample **solution** in the presence of aminolevulinic acid dehydratase and subsequently incubating the sample **solution** in the presence of the substrate.

19. The lead assay of claim 5 wherein step (d) includes incubating the sample **solution** in the presence of a coloring reagent.

21. An aqueous lead assay reagent **solution** consisting essentially of neutralizing buffer and a lead chelator having a lead binding constant in the range of about 4 log K to about 13 log K wherein the concentration of the chelator in the **solution** is in the range of 0.5 mM to 500 mM.

22. The reagent **solution** of claim 21 wherein the chelator has a lead equilibrium binding constant in the range of about 6 log K. .

.

23. The reagent **solution** of claim 21 wherein the chelator is selected from the group consisting of N-benzyiminodiacetic acid, ethylenebis(oxyethylenenitrilo)tetraacetic acid, ethylenediaminetetraacetic acid, L-histidine. . .

24. The reagent **solution** of claim 21 wherein the chelator is selected from the group consisting of 8-hydroxy-5-(2'-hydroxyphenylazo)quinoline, 8-hydroxy-5-(phenylazo)quinoline, N-(2-carboxyphenyl)iminodiacetic acid, N-(acetonyl)iminodiacetic acid, N-(dithiocarboxy)aminoacetic. . .

25. The reagent **solution** of claim 21 wherein the chelator is selected from the group consisting of dihydroxyphenyl acetic acid, N-(2'-carboxyethyl)iminodiacetic acid, dihydroxybenzoic acid,. . .

26. A lead assay reagent kit comprising: a container having a reagent **solution** consisting essentially of an aqueous neutralizing buffer and present therein a reagent which is capable of forming a compound or complex with lead such that adding the **solution** to an acidified aqueous sample containing lead will neutralize the aqueous sample while preventing precipitation of lead therefrom; a container.

.

.

IT 52-66-4, DL-Penicillamine **60-00-4**, EDTA, uses 67-42-5, EGTA 71-00-1, L-Histidine, uses 81-88-9 84-88-8, 8-Hydroxyquinoline-5-sulfonic acid 93-62-9, N-(2-Hydroxyethyl)-iminodiacetic acid **106-60-5**, Aminolevulinic acid 139-13-9, Nitrilotriacetic acid 142-73-4, Iminodiacetic acid 487-90-1, Porphobilinogen 994-36-5, Sodium citrate 3987-53-9, N-Benzyliminodiacetic acid 4408-64-4, Methyliminodiacetic acid 5961-85-3, Tris(2-carboxyethyl)phosphine **9036-37-7**
(automated lead assay in blood using disulfide enzyme)

L18 ANSWER 3 OF 6 USPATFULL
ACCESSION NUMBER: 97:7810 USPATFULL
TITLE: Automated lead assay

INVENTOR(S): Wong, Martin, Grayslake, IL, United States
Finley, David M., Spring Grove, IL, United States
Ramp, John M., Gurnee, IL, United States
Boltinghouse, Jr., Gary L., McHenry, IL, United States
Shaffar, Mark R., Kenosha, WI, United States
Stroupe, Stephen D., Libertyville, IL, United States
Brackett, John M., Kenosha, WI, United States
PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5597702		19970128
APPLICATION INFO.:	US 1994-350241		19941209 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1993-171121, filed on 21 Dec 1993, now abandoned And a continuation-in-part of Ser. No. US 1993-171035, filed on 21 Dec 1993, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Schain, Howard E.
ASSISTANT EXAMINER: Mohamed, Abdel A.
LEGAL REPRESENTATIVE: Weinstein, David L.
NUMBER OF CLAIMS: 27
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 5 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT: 1558
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Detection of lead present in a sample, comprising the steps of: (a) adding a lead recovery agent to an assay **solution** containing lead from the sample; (b) adding to the assay **solution** a disulfide enzyme which is inhibited in the presence of lead; and (c) correlating the activity of the disulfide enzyme. . .

SUMM . . . Unfortunately, these compounds interfere with color development of the porphobilinogen reaction product and hence must be precipitated from the assay **solution**. Known compounds typically used for effecting such precipitation are mercury salts which are unattractive from an environmental and toxicological standpoint.. . . desirable if a non mercury-based reducing agent could be discovered which would not need to be precipitated from the assay **solution**.

SUMM . . . blood, the assay comprising the steps of (i) adding an aminolevulinic acid dehydratase enzyme and aminolevulinic acid to an assay **solution** comprising a supernatant separated from the blood sample, wherein the supernatant, at the time of said addition, has a neutral. . .

SUMM . . . further method according to the invention, lead is detected in a sample suspected of containing lead by forming an assay **solution** in which there is combined a sample suspected of containing lead and an enzyme which is inhibited in the presence. . . and a fluorescer (i.e., fluorophor) which does not react chemically with the enzyme, the substrate or the reaction product. This **solution** then is incubated under conditions sufficient to produce the reaction product. The incubated assay **solution** is then treated with a coloring reagent to convert the reaction product to a chromophore capable of providing a change in the transmitive properties of the assay

solution within a wavelength band that overlaps the excitation and/or emission wavelength band of the fluorescer. After the coloring step, the assay **solution** is irradiated with light having a wavelength within the excitation wavelength band of the fluorescer, and the fluorescence emitted by the assay **solution** is then detected and measured as a means of measuring the concentration of lead in the sample. This method can. . .

DETD The enzyme activity in the assay **solution** can be measured in a number of ways by determining the amount of the substrate utilized or the PBG produced. Other reactants can be added to the sample **solution** for a subsequent reaction therewith. For example, an assay can be performed by competitively binding a suitable antibody to the. . .

DETD . . . time making the lead available for interaction with disulfide enzyme. The term "neutralizing reagent" or "neutralizing buffer" refers to the **solution** which brings the above-described acidified whole blood supernatant sample to a neutral pH. The lead recovery agents of the present. . .

DETD . . . The sample suspected of containing lead is first treated in a conventional manner to isolate the lead into an aqueous **solution** so that the lead is separated from other compounds or substances originally in the untreated sample which may interfere with. . .

DETD . . . the sample has been contacted with the lead recovery agent, the assay continues by incubating the enzyme with the sample **solution** in the presence of a substrate. The enzyme incubation step is stopped after a predetermined time interval. In the case. . .

DETD . . . is the ability to obtain a marked reduction in interference from other metals which may be present in the assay **solution**. The table shown below reports the results of our investigations of metal interference in the assay of the present invention. . .

DETD . . . with a mercury salt (mercuric chloride). Mercury forms a precipitate with the DTT which can be removed from the assay **solution** by centrifugation or the like.

DETD . . . do not interfere with the PBG color development, and hence do not have to be precipitated from the incubated assay **solution**. In particular, we have discovered that water-soluble tertiary phosphines can be used to enhance the reaction between disulfide enzymes and. . .

DETD . . . enhancing agents which we have found can be substituted for mercuric ion in either the stop reagent or the colorant **solution** or both. A preferred color enhancing agent comprises the cupric ion Cu.sup.+2. Any compound which contains the cupric ion is. . .

DETD . . . precipitation. A suitable concentration range for the cupric ion is about 1 mM to about 500 mM in the assay **solution**.

DETD . . . DTT. A preferred concentration range for the ferric ion is about 1 mM to about 500 mM in the assay **solution**.

DETD The ferric or cupric compounds can be added to the sample **solution** prior to photometrically determining the extent of the incubation reaction. The addition can take place while adding the stopping reagent. . .

DETD In particular, after the coloring step in which porphobilinogen is reacted with a coloring agent, the assay **solution** is irradiated with light having a wavelength within the excitation wavelength band of the fluorescer, and the fluorescence emitted by the assay **solution** is then detected and measured as a means of measuring the concentration of lead in the assay reaction mixture. The.

. . fluorometric analysis. Other dyes may be used provided they are compatible with the pH and other conditions of the assay **solution**.

DETD . . . grade distilled water was adjusted to pH 1.50 by adding an appropriate amount of concentrated HNO.sub.3. A 20 mM ZnCl.sub.2 **solution** was prepared by adding pH 1.50 distilled water to 0.0340 g ZnCl.sub.2 for a final **solution** weight of 12.500 g. The **solution** was then thoroughly mixed.

DETD A **solution** of 200 ml 1.5M Bis-Tris was prepared by adding 62.70 g of Bis-Tris to HPLC grade distilled water to a. . . to 7.30 with concentrated HNO.sub.3. The resulting volume was adjusted to the mark with distilled water. Similarly a 200 ml **solution** of 2.0M Bis-Tris was prepared by using 83.60 g of Bis-Tris. The pH was adjusted to pH 7.60 before adjusting. . .

DETD . . . adding 5 ml of ALAD containing 3.1 U/mg to 35 ml of 250 mM Bis-Tris. The 250 mM Bis-Tris diluent **solution** was prepared by adding 5.23 g. Bis-Tris to 100 ml of HPLC grade distilled water and stirring. DTT was added. . . such glutathione, mercaptoethanol and cysteine can be used as a reducing agent instead of DTT. The pH of the diluent **solution** was adjusted to pH 7.0 by adding 50% NaOH. The diluted enzyme reagent was stored at 2.degree.-8.degree. C. under nitrogen. . .

DETD A 25 mM ALA and 10 .mu.M ZnCl.sub.2 substrate **solution** was prepared by adding 0.210 g. ALA, 25 .mu.l 20 mM ZnCl.sub.2 and 50 ml HPLC distilled water to a flask. After stirring, the substrate **solution** was stored at 2.8.degree. C. in the dark.

DETD . . . TCA was prepared by adding 20.000 g of TCA, 0.1M HgCl.sub.2 and

HPLC grade distilled water to 200 ml. The **solution** was stirred and filtered at 0.80 um.

DETD For each lead recovery agent, a neutralizing **solution** containing 0.5M of the agent and 1.5M Bis-Tris was prepared by adding 7.5 ml of the 2M Bis-Tris **solution** to following amounts of recovery agents: Na Citrate 1.470 gm; IDA 0.975 g; NTA 1.175 g; EGTA 1.900 g; Histidine. . . 1.125 g; EDTA 1.840 g; and PEN 0.745 g. After

stirring, HPLC grade distilled water was added to each neutralizing **solution** to obtain a final volume of about 9.5 ml. Subsequently, the recovery agent solutions were vigorously stirred overnight. The neutralizing. . . were heated to about 70.degree. C. to complete dissolution and then cooled to room temperature. The pH of each neutralizing **solution** was then adjusted to pH 7.25 with either concentrated HNO3 or 50% NaOH.

DETD . . . and 40 .mu.g/dl Pb.sup.+2 were dispensed in 24.5 ml amounts and

were pretreated with 10.5 ml of the TCA pretreatment **solution**.

DETD Each sample was centrifuged for five minutes and the supernatants were saved. From each supernatant **solution** 180 .mu.l was mixed by vortex with 180 .mu.l of neutralizing buffer. From this neutralized supernatant **solution** 100 ul was added to 100 .mu.l of the dilute enzyme reagent and mixed by vortex and incubated for 15. . .

DETD Subsequently 100 .mu.l of the substrate **solution** was added, mixed by vortex and incubated for 30 min. in the water bath. The stop reagent was added in. . .

DETD . . . appropriate amount of concentrated HNO3. The pH 1.50 distilled water is then added to 0.0340 g. ZnCl.sub.2 for a final **solution** weight of 12.500 g. The **solution** is then thoroughly mixed.

DETD A neutralizing **solution** containing 0.5M IDA, 0.125M Histidine and 1.5M Bis-Tris is prepared by adding 7.5 ml of a 2M Bis-Tris **solution** to following amounts of IDA 0.975 g and Histidine 1.050

g. After stirring, HPLC grade distilled water is added to the neutralizing **solution** to obtain a final volume of about 9.5 ml. A **solution** of 200 ml 2M Bis-Tris was prepared by adding 83.60 g of Bis-Tris to HPLC grade distilled water to a. . . is adjusted to 7.11 with concentrated HNO.sub.3. The resulting volume is adjusted to 200 ml with distilled water. The Bis-Tris **solution** is stirred for 10 min. at room temperature and filtered to remove any visible particles.

DETD . . . adding 5 ml of ALAD containing 3.1 U/mg to 35 ml of 250 mM Bis-Tris. The 250 mM Bis-Tris diluent **solution** is prepared by adding 5.23 g Bis-Tris to 100 ml of HPLC grade distilled water and stirring. DTT is added to 15 mM in the diluted enzyme reagent. The pH of the diluent **solution** is adjusted to pH 7.0 by adding 50% NaOH. The diluted enzyme reagent is stored at 2.degree.-8.degree. C. under nitrogen. . .

DETD A 25 mM ALA and 10 .mu.M ZnCl.sub.2 substrate **solution** is prepared by adding 0.0127 g. ALA, 30 ul 1 mM ZnCl.sub.2 and 3 ml HPLC distilled water to a flask. After stirring, the substrate **solution** is stored at 2.8.degree. C. in the dark.

DETD . . . TCA is prepared by adding 20.000 g of TCA, 0.1M HgCl.sub.2 and HPLC grade distilled water to 200 ml. The **solution** is stirred and filtered at 0.80 .mu.m. The various concentrations reported in Table 1 below are prepared by serial dilutions.

DETD Subsequently 100 .mu.l of the substrate **solution** is added, mixed by vortexing and incubated for 25 min. in the water bath. The stop reagent is added in. . .

DETD . . . at 0, 7, 14, 21, 28 and 42 .mu.g/dL were prepared by gravimetric dilution from a 10 mg/dL stock lead **solution** into an aqueous **solution** of 75 mM citric acid adjusted to pH 0.95.

DETD A 1.5M Bis-Tris **solution** in HPLC distilled water is prepared and adjusted to pH 7.3 with concentrated HNO.sub.3. The **solution** was filtered to remove particulates and maintained at room temperature.

DETD A stock **solution** of 100 mM HQSA in the 1.5M Bis-Tris **solution** is prepared and adjusted to pH 7.25.

DETD 1. Neutralizing Buffer. Neutralizing buffer was prepared by adding 10 ml of the above 100 mM HQSA stock **solution** to 90 ml of the 1.5M Bis-Tris **solution**. The pH is adjusted to 7.30 and the **solution** is maintained at room temperature. The neutralizing buffer is placed in the first reagent well of an IMx.RTM. reagent pack. . .

DETD 2. ALAD Enzyme Reagent. ALAD enzyme reagent is prepared by diluting one part 3.1 U/mg ALAD **solution** into 3 parts 150 mM Bis-Tris **solution** then adding Rhodamine 110 to a final concentration of 5 .mu.M. The ALAD enzyme reagent is placed in the second. . .

DETD 3. Substrate Reagent. A substrate **solution** in HPLC distilled water is prepared containing 40 mM ALA, 20 .mu.m ZnCl, and 20 mM TCEP and 5 .mu.M. . .

DETD . . . cartridge and the cuvette, is performed by the IMx robotic pipetting arm. Fluorescence intensity readings are taken on the assay **solution** in the IMx cuvette using the IMX FPIA optical assembly without polarization. The reagent pack, reagent wells, sample cartridge, sample. . .

DETD 8. The assay **solution** present in the cuvette (neutralized sample, ALAD enzyme reagent and phosphate dilution buffer) is permitted to incubate for 5.67 minutes.

DETD 13. A reading is taken of fluorescence intensity of the assay **solution** in the cuvette using the optical equipment of the IMx.RTM. at 485 nm for excitation and 525 nm for emission.

DETD . . . mM HQSA, pH 7.60. ALAD Enzyme Reagent is prepared at pH 7.10 by diluting 5.8 U/mg ALAD 1/4 into a **solution** containing 250 mM Bis-Tris, 0.5% PEG 8000, 0.2% sodium azide and 5 .mu.M rhodamine 116. Substrate reagent is prepared in. . .

DETD . . . at 0, 7, 14, 21, 28 and 42 .mu.g/dL were prepared by gravimetric dilution from a 10 mg/dL stock lead **solution** into 75 mM citric acid at pH 0.90. The actual lead concentrations of the samples as determined by atomic absorption. . .

DETD 8. The assay **solution** present in the cuvette (neutralized sample, ALAD enzyme reagent and TDx.RTM. dilution buffer) is permitted to incubate for 6.25 minutes.

DETD 10. The assay **solution** now present in the cuvette (ALAD enzyme reagent, substrate reagent, neutralized sample, and TDx.RTM. buffer) is permitted to incubate for. . .

DETD 13. A reading is taken of fluorescence intensity of the assay **solution** in the cuvette using the optical equipment of the IMx.RTM. at 485 nm for excitation and 525 nm for emission.

DETD . . . lead standards at concentrations of 0, 7, 14 and 28 .mu.g/dl were prepared by dilution from an 8 mg/dl stock **solution** of lead nitrate nitrate. The dilutions were carried out using HPLC deionized water adjusted to a pH of 2.0 with. . .

DETD . . . Aqueous neutralizing buffer: An aqueous neutralizing buffer is prepared containing 1.5M Bis-Tris, 0.5M IDA, 0.125M histidine. The pH of the **solution** was adjusted to 7.05 with concentrated nitric acid.

DETD 4. ALAD enzyme **solution**: A buffer **solution** is first prepared by dissolving 10.08 grams of Bis-Tris, 21.40 grams of sucrose in 250 ml water. DTT was added to the **solution** to obtain a concentration of 6.25 mM. The **solution** was then adjusted to pH 6.9 with nitric acid and allowed to incubate for about 90 minutes at room temperature. ALAD was diluted 1/5 (by weight) into this buffer. The ALAD **solution** is then adjusted to pH 7.1.

DETD 5. ALA substrate **solution**: An aqueous **solution** of ALA containing 0.25M Bis-Tris, 0.125M sucrose, 10 mM ALA, and 2.5 mM glutathione is prepared by combining 5.22 g. . .

DETD 6. Stop buffer. A **solution** was prepared containing 10% TCA and 0.1M mercuric chloride. A stop buffer is prepared by combining 5.01 grams of TCA and 1.36 grams mercuric chloride in sufficient water to bring the total weight of the **solution** to 53.28 grams.

DETD (a) 50 .mu.l of the neutralized standard **solution** are added to the sample well C of the test cartridge. The test cartridges are then loaded onto the centrifuge. . . was stopped and reagents were manually added to the test pack as follows: (i) 50 .mu.l of the ALAD enzyme **solution**, prepared above, is placed in the reagent well A of the test cartridge; (ii) 50 .mu.l of the substrate **solution** is placed in the sample well C of the test cartridge; and (iii) 110 .mu.l of the stop buffer is. . .

DETD . . . pre-programmed to perform a mix-install cycle having duration of 5 and 3 seconds respectively. This mix-install cycle mixes the enzyme **solution** in reagent well A with the sample that is already present in the reading chamber in step (a) according to. . .

DETD Thus, at the end of the mix-install cycle performed in this step (c), the enzyme **solution** has been mixed with the original sample

and is now present in the reading chamber H along with the sample; the substrate **solution** has not yet combined with the enzyme sample mixture but is now positioned in region F; and the stop buffer. . .

DETD . . . pre-programmed to retain the test cartridge in the install position for a period of 10 minutes to allow the sample **solution** and the enzyme **solution** to incubate in the reading well H.

DETD . . . another mix-install cycle of 5 seconds (mix) and 1800 seconds (install). This mix-install cycle mixes the substrate with the sample/enzyme **solution** and permits a 30 minute incubation. The pathways for the substrate and the enzyme/sample mixture are as follows:

##STR3##

DETD At the conclusion of this step (e) the assay **solution** present in the reading chamber has incubated 30 minutes and now contains the ALAD/ALA reaction product porphobilinogen.

DETD . . . manually added to reagent well A. Several mix install cycles are performed to mix the Ehrlich's reagent with the assay **solution**. At the conclusion of this step, the colored assay **solution** is present in the reading chamber H of the test cartridge.

CLM What is claimed is:

. . . blood, the assay comprising the steps of (i) adding an aminolevulinic acid dehydratase enzyme and aminolevulinic acid to an assay **solution** comprising a supernatant separated from the whole blood sample, wherein the supernatant, at the time of said addition, has a.

.

23. A lead assay comprising the steps of: a) forming an assay **solution** by combining a sample suspected of containing lead with (i) an enzyme which is inhibited in the presence of lead;. . . a fluorescer which does not react chemically with the enzyme, the substrate or the reaction product; b) incubating the assay **solution** under conditions sufficient to produce said reaction product; c) contacting the assay **solution** with a coloring reagent capable of converting said reaction product to a chromophore capable of changing the transmissive properties of the assay **solution** within a wavelength band that overlaps the excitation and/or emission wavelength band of the fluorescer; d) irradiating the assay **solution** with light having a wavelength within the excitation wavelength band of the fluorescer; (e) measuring the fluorescence emitted by the assay **solution** as a measure of the concentration of lead in the sample.

25. The method of claim 24 wherein the sample is a supernatant obtained from whole blood and said assay **solution** further comprises a lead recovery agent and a tertiary phosphine.

IT **9036-37-7**, .delta.-Aminolevulinate dehydratase
(automated lead assay)

IT 52-66-4, DL-Penicillamine **60-00-4**, Ethylenediaminetetraacetic acid, uses 67-42-5, Ethylenebis(oxyethylenenitrilo)tetraacetic acid 68-04-2, Sodium citrate 84-88-8, 8-Hydroxyquinoline-5-sulfonic acid 93-62-9, N-(2-Hydroxyethyl)iminodiacetic acid 139-13-9, Nitrilotriacetic acid 142-73-4, Iminodiacetic acid 645-35-2, L-Histidine monohydrochloride 3987-53-9, N-Benzyliminodiacetic acid 4408-64-4, Methyliminodiacetic acid
(lead recovery agent; automated lead assay)

IT **106-60-5**, .delta.-Aminolevulinic acid
(substrate; automated lead assay)

L18 ANSWER 4 OF 6 USPATFULL

ACCESSION NUMBER: 96:80166 USPATFULL

TITLE: Lead detection method and reggents utilizing aminolevulinic acid dehydratase and tertiary phosphines

INVENTOR(S): Wong, Martin, Grayslake, IL, United States
Ramp, John M., Gurnee, IL, United States
Brackett, John M., Kenosha, WI, United States

PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5552297		19960903
APPLICATION INFO.:	US 1995-419845		19950411 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-171035, filed on 21 Dec 1993, now abandoned

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Gitomer, Ralph J.
LEGAL REPRESENTATIVE: Levis, John F.
NUMBER OF CLAIMS: 36
EXEMPLARY CLAIM: 1
LINE COUNT: 827

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method and kit for simplifying and improving the sensitivity and accuracy of a lead assay for a sample **solution** suspected of containing lead determines the extent of a reaction between a substrate and a disulfide enzyme in the presence. . . a colorimetric determination of the enzyme activity a chromophore is formed upon reaction with a selected component of the sample **solution** in the presence of a colorimetric enhancing reagent. The colorimetric enhancing reagent contains a metal ion such as cupric ion or ferric ion which is soluble in the sample **solution**. The extent of the chromophore formation is then photometrically determined.

SUMM . . . demonstrating lead as a noncompetitive inhibitor of ALAD activity. The incubation mixtures contained DTT, ALAD and ALA in a buffer **solution**. The incubations were terminated by the addition of TCA which also contained HgCl.sub.2. The **solution** was centrifuged and the supernatant was added to modified Ehrlich's reagent in acetic acid and perchloric acid. The colored complex. . .

SUMM A significant problem in using an ALAD assay is the toxicity of the mercury used in the TCA **solution** as well as in the modified Ehrlich's reagent to eliminate interference with chromophore formation by sulfhydryl compounds. The disposal of. . .

SUMM Another **solution** to the problem is to eliminate using mercury altogether. One inventive approach is to replace the prior art sulfhydryl compounds. . .

SUMM The present invention provides a method of improving the sensitivity and accuracy of a lead assay for a sample **solution** suspected of containing lead. The assay determines the extent of a reaction between a substrate and a disulfide enzyme. The. . .

SUMM . . . of improving the sensitivity and accuracy of a lead assay includes incubating an aminolevulinic acid dehydratase enzyme in a sample **solution** suspected of containing lead in the presence of a water-soluble tertiary phosphine and a substrate containing

aminolevulinic acid to form. . .

SUMM Another method contemplated by the present invention includes incubating a disulfide enzyme in a sample **solution** suspected of containing lead in the presence of a reducing reagent and a substrate to form a reaction product. The. . . stopped after a predetermined time interval and a chromophore is formed upon reaction with a selected component of the sample **solution** in the presence of a colorimetric enhancing reagent. The colorimetric enhancing reagent contains a metal ion soluble in the sample **solution** which is selected from the group consisting essentially of a cupric ion and a ferric ion. The extent of the. . .

SUMM . . . activity of a disulfide enzyme in a lead assay. The colorimetric enhancing reagent contains a metal ion soluble in a **solution** containing the product of the enzyme activity. The metal ion is selected from the group consisting essentially of a cupric.

. .

SUMM . . . The stop reagent also commonly contains mercury compound such as HgCl.sub.2 to release the Hg.sup.+2 metal ion in the sample **solution**.

SUMM Adding the stop reagent to the sample **solution** produces a precipitate which potentially interferes with the determination of the amount of the product resulting from the assay which. . . extent of the enzyme and substrate reaction is determined. Centrifugation is commonly used to remove the precipitate from the sample **solution** . The resulting supernatant contains the PBG which is then separated from the precipitate for further processing.

SUMM A colorimetric determination of the reaction product is widely used. In this technique, Ehrlich's reagent is added to the sample **solution** after the supernatant is separated from the precipitate to form a chromophore upon reaction with the PBG. Often, the Ehrlich's reagent is modified to contain a mercury compound to provide a Hg.sup.+2 metal ion in the sample **solution** which precipitates sulfhydryl compounds interfering with the chromophore formation. The precipitation of the sulfhydryl compounds by the mercuric ion improves. . .

SUMM . . . the chromophore reaction of the assay. Furthermore, the inventive activating reagents do not need to be removed from the sample **solution** by forming a precipitate. Thus, two entire steps of the prior art assay are eliminated. Since the present invention avoids forming a precipitate, there is no need to centrifuge the sample **solution** and separate the reaction product contained in the supernatant for further processing.

SUMM . . . to determine the extent of the incubation reaction between the disulfide enzyme and the substrate. Any component of the sample **solution** can be directly analyzed after the incubation reaction has been stopped. For example, this includes measuring the amount of the. . .

SUMM . . . determination of the amount of the substrate utilized or the PBG produced. Other reactants can be added to the sample **solution** for a subsequent reaction therewith. For example, an assay can be performed by competitively binding a suitable antibody to the. . .

SUMM . . . also found certain colorimetric enhancing reagents can be substituted for the mercuric ion in either the stop reagent or Ehrlich's **solution** or both. These inventive colorimetric enhancing reagents are relatively less toxic and environmentally hazardous than

the mercuric ion.

SUMM A coloring reagent is added to the sample **solution** to form a chromophore upon reaction with the product or other preselected reactant found in the sample **solution**. Suitable coloring reagents for use in the present invention include dimethylaminobenzaldehyde, dimethylaminocinnamaldehyde, or their derivatives.

SUMM The inventive colorimetric enhancing reagents are added to the sample **solution** prior to photometrically determining the extent of the incubation reaction. The addition can take place while adding the stopping reagent. . .

SUMM . . . can continue with incubation of the ALAD enzyme. Subsequently, the assay continues by incubating an ALAD enzyme in the sample **solution** in the presence of a substrate such as ALA.

DETD . . . appropriate amount of concentrated HNO.sub.3. The pH 1.50 distilled water was then added to 0.0340 g. ZnCl.sub.2 for a final **solution** weight of 12,500 g. The **solution** was then thoroughly mixed.

DETD A neutralizing **solution** containing 0.5M IDA, 0.125M Histidine and 1.5M BisTris was prepared by adding 7.5 ml of a 2M Bis-Tris **solution** to following amounts of IDA 0.975 g and Histidine 1.050 g. After stirring, HPLC grade distilled water was added to each neutralizing **solution** to obtain a final volume of about 9.5 ml. A **solution** of 200 ml 2M BisTris was prepared by adding 83.60 g of BisTris to HPLC grade distilled water to a. . . was adjusted to 7.11 with concentrated HNO.sub.3. The resulting volume was adjusted to 200 ml with distilled water. The BisTris **solution** was stirred for 10 min. at room temperature and filtered to remove any visible particles.

DETD . . . adding 5 ml of ALAD containing 3.1 U/mg to 35 ml of 250 mM BisTris. The 250 mM BisTris diluent **solution** was prepared by adding 5.23 g. BisTris to 100 ml of HPLC grade distilled water and stirring. DTT was added to 15 mM in the diluted enzyme reagent. The pH of the diluent **solution** was adjusted to pH 7.0 by adding 50% NaOH. The diluted enzyme reagent was stored at 2.degree.-8.degree. C. under nitrogen. . .

DETD A 25 mM ALA and 10 uM ZnCl.sub.2 substrate **solution** was prepared by adding 0.0127 g. ALA, 30 ul 1 mM ZnCl.sub.2 and 3 ml HPLC distilled water to a flask. After stirring, the substrate **solution** was stored at 2.8.degree. C. in the dark.

DETD . . . TCA was prepared by adding 20.000 g of TCA, 0.1M HgCl.sub.2 and HPLC grade distilled water to 200 ml. The **solution** was stirred and filtered at 0.80 um. The various concentrations reported in Table 1 below were prepared by serial dilutions.

DETD Subsequently 100 ul of the substrate **solution** was added, mixed by vortexing and incubated for 25 min. in the water bath. The stop reagent was added in. . .

DETD . . . the substrate at the time of incubation. The activating reagent may be kept separate or added to either the enzyme **solution** or the sample containing the ALAD enzyme.

CLM What is claimed is:

. . . 1. A method for detecting lead in a sample suspected of containing lead, the method comprising: (a) forming an aqueous **solution** from the sample such that any lead in the sample is present in said aqueous **solution**; (b) contacting the aqueous **solution** with an aminolevulinic acid dehydratase enzyme in the presence of a water soluble tertiary phosphine; (c) incubating the enzyme with. . . . . in the presence of a colorimetric enhancing reagent, said

colorimetric enhancing reagent comprising a cupric ion soluble in the aqueous **solution**.

5. The method of claim 4 wherein the colorimetric enhancing reagent is added directly to the aqueous **solution** after incubation of the aminolevulinic acid dehydratase and aminolevulinic acid in step (c).

6. The method of claim 1 wherein the method comprises acidifying the aqueous **solution** to isolate the lead from compounds which interfere with said method, said compounds being selected from the group

consisting of proteins, endogenous d-aminolevulinic acid dehydratase, porphobilinogen and aminolevulinic acid, and neutralizing the aqueous **solution** before said enzyme incubation of step (c).

7. The method of claim 1 wherein the stopping step includes acidifying the aqueous **solution** and adding a coloring reagent to form a chromophore upon reaction with said porphobilinogen.

. . . in the presence of a colorimetric enhancing reagent, the colorimetric

enhancing reagent comprising a cupric ion soluble in the aqueous **solution**; and wherein step (e) includes photometrically detecting said chromophore.

. . . 11. A method for detecting lead in a sample suspected of containing lead, the method comprising: (a) forming an aqueous **solution** from the sample such that any lead in the sample is present in said aqueous **solution**; (b) incubating in said aqueous **solution**, (i) an aminolevulinic acid dehydratase enzyme; and (ii) aminolevulinic acid, in the presence of a reducing agent, to form porphobilinogen;. . .

12. The method of claim 11 wherein the method further includes acidifying the aqueous **solution** to isolate the lead in the **solution** from compounds which interfere with said method, said compounds being selected from the group consisting of proteins, endogenous d-aminolevulinic acid dehydratase, porphobilinogen and aminolevulinic acid, and neutralizing the aqueous **solution** before said enzyme incubation of step (b).

13. The method of claim 11 wherein step (c) includes acidifying the aqueous **solution** and adding a coloring reagent to the **solution**.

IT 52-66-4, DL-Penicillamine **60-00-4**, EDTA, uses 67-42-5, EGTA 71-00-1, L-Histidine, uses 81-88-9 84-88-8, 8-Hydroxyquinoline-5-sulfonic acid 93-62-9, N-(2-Hydroxyethyl)-iminodiacetic acid **106-60-5**, Aminolevulinic acid 139-13-9, Nitrilotriacetic acid 142-73-4, Iminodiacetic acid 487-90-1, Porphobilinogen 994-36-5, Sodium citrate 3987-53-9, N-Benzyliminodiacetic acid 4408-64-4, Methyliminodiacetic acid 5961-85-3, Tris(2-carboxyethyl)phosphine **9036-37-7**
(automated lead assay in blood using disulfide enzyme)

L18 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:780833 CAPLUS
DOCUMENT NUMBER: 130:257240
TITLE: Stability of 5-aminolevulinic acid in aqueous **solution**
AUTHOR(S): Elfsson, B.; Wallin, I.; Eksborg, S.; Rudaeus, K.;

Ros, A. M.; Ehrsson, H.
CORPORATE SOURCE: Karolinska Pharmacy, Stockholm, S-171 76, Swed.
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(4) Chang, S; J Urol 1996, V155, P1744 CAPLUS
(7) Edwards, S; Neuropharmacology 1984, V23, P477 CAPLUS
(8) Fijan, S; Br J Dermatol 1995, V133, P282 CAPLUS
(9) Franck, B; Heterocycles 1981, V15, P919 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Stability of 5-aminolevulinic acid in aqueous **solution**
IT **60-00-4**, EDTA, properties
RL: PRP (Properties)
(stability of aminolevulinic acid in aq. soln.)
IT **106-60-5**, 5-Aminolevulinic acid
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study);
USES
(Uses)
(stability of aminolevulinic acid in aq. soln.)

L18 ANSWER 6 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1999:181462 BIOSIS
DOCUMENT NUMBER: PREV199900181462
TITLE: Stability of 5-aminolevulinic acid in aqueous **solution**.
AUTHOR(S): Elfsson, B.; Wallin, I.; Eksborg, S.; Rudaeus, K.; Ros, A. M.; Ehrsson, H. (1)
CORPORATE SOURCE: (1) Karolinska Pharmacy, S-171 76, Stockholm Sweden
SOURCE: European Journal of Pharmaceutical Sciences, (Jan., 1999) Vol. 7, No. 2, pp. 87-91.
ISSN: 0928-0987.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Stability of 5-aminolevulinic acid in aqueous **solution**.
AB The chemical stability of 5-aminolevulinic acid (ALA) was studied in aqueous **solution** as a function of concentration, pH, temperature and in the presence of ethylenediaminetetraacetic acid (EDTA). The degradation of ALA was. . .
IT Major Concepts
Pharmacology
IT Chemicals & Biochemicals
ethylenediaminetetraacetic acid; 5-aminolevulinic acid: aqueous **solution**, stability
RN **106-60-5** (5-AMINOLEVULINIC ACID)
60-00-4 (EDTA)